

# Effect of ormeloxifene, a nonsteroidal once-a-week oral contraceptive, on systemic hemodynamics in adult female rats

Hiralal Bhalla, Kamlesh Kumar Pant<sup>1</sup>, Madhu Dikshit<sup>2</sup>, William R. Surin<sup>2</sup>, Man Mohan Singh<sup>3</sup>

Department of Pharmacology, Subharti Medical College, Meerut, <sup>1</sup>Department of Pharmacology and Therapeutics, CSM Medical University, Lucknow, <sup>2</sup>Department of Pharmacology, Central Drug Research Institute, Lucknow, <sup>3</sup>Saraswati Dental College and Hospital, Faizabad Road, Lucknow, India

## ABSTRACT

**Objective:** To investigate the short-term effects of ormeloxifene on systemic hemodynamics, coagulation profile, and serum antioxidant activity *in vivo* in comparison with raloxifene. **Materials and Methods:** Colony-bred adult female Sprague-Dawley rats were randomized into 19 groups of 10 each and received either ormeloxifene or raloxifene (0.25, 1.25, or 3 mg/kg/day) for 7, 15, or 30 days by the oral route. Animals of control group received vehicle (gum-acacia in distilled water) alone in a similar manner. Systemic hemodynamics and serum total antioxidant activity were assessed 24 h after the last treatment. **Results:** There was no significant effect of ormeloxifene administered at these doses and schedules on hemodynamic parameters or antioxidant activity, except for increase in amplitude of R wave in rats treated with 3 mg/kg/day dose for 30 days. This effect with raloxifene was evident only 7 days after treatment at this dose. Overall response was, however, almost similar with both the agents. **Conclusion:** The findings demonstrate comparable pharmacological profile of ormeloxifene and raloxifene on short-term administration to rats. Based on changes observed in the ECG (R wave), long-term studies may lead to justifiable comparison of beneficial and harmful effects of ormeloxifene and raloxifene in relation to cardiovascular effects.

**Key words:** Toxicity, rodents, NOAEL

## INTRODUCTION

Atherosclerotic vascular disease is the major cause of morbidity and mortality in postmenopausal women. Hormone replacement therapy (HRT), though indicated as cardio-protective,<sup>[1]</sup> is associated with an increased risk of endometrial hyperplasia, carcinoma,<sup>[2]</sup> breast cancer,<sup>[3]</sup> and thromboembolism.<sup>[4]</sup> Efforts

have been made to develop agents that prevent estrogen-deficiency-related clinical disorders and are free from estrogen-related health hazards.

Selective estrogen receptor modulators (SERM), by virtue of their tissue-selective pharmacology,<sup>[5]</sup> have attracted attention of researchers in recent years. Ormeloxifene is one such multifunctional nonsteroidal SERM possessing potent estrogen-antagonistic and weak estrogen-agonistic activities and has been marketed in India as once-a-week oral contraceptive.<sup>[6]</sup> It prevents ovariectomy-induced bone loss<sup>[7]</sup> by inhibiting generation and activity of osteoclasts.<sup>[8]</sup> In women, ormeloxifene (30 mg/week) administered for 1 year does not cause hyperaggregability of platelets. Lack of effect of ormeloxifene (up to 80 mg/kg) on vascular cyclooxygenase activity in rats at concentrations inhibiting malonaldehyde

| Access this article online                                                          |                                                                        |
|-------------------------------------------------------------------------------------|------------------------------------------------------------------------|
| Quick Response Code:                                                                | Website:<br><a href="http://www.jpharmacol.com">www.jpharmacol.com</a> |
|  | DOI:<br>10.4103/0976-500X.81898                                        |

### Address for correspondence:

Hiralal Bhalla, Department of Pharmacology, Subharti Medical College, Meerut, India. E-mail: [hirabhalla2010@gmail.com](mailto:hirabhalla2010@gmail.com)

synthesis suggest sparing effect of ormeloxifene that might prove beneficial against risk of thrombotic episodes by shift of balance towards more beneficial antiaggregatory prostaglandin- $I_2$ .<sup>[5,9]</sup> Raloxifene, that mimics effects of estrogen in bone and cardiovascular system and exhibits estrogen-antagonistic effect on endometrium,<sup>[10]</sup> increases incidence of hot flushes, deep vein thrombosis, pulmonary embolism and leg cramps in women similar to that associated with HRT use and exhibit potent estrogenic responses in animals.<sup>[7]</sup> This study investigates short-term effects of ormeloxifene on systemic hemodynamics, coagulation profile, and serum antioxidant activity in comparison to raloxifene in adult female rats, no information on which is available.

## MATERIALS AND METHODS

### Animals

Colony-bred adult (180 -200 g) virgin female Sprague-Dawley rats maintained under standard conditions with alternate 12 h light/dark periods and free access to pellet diet and tap water as per CPCSEA guidelines were used. The study protocol was approved by the Institutional Animal Ethics Committee.

### Chemicals

Raloxifene was purchased (Ralista-Cipla Ltd., India) and ormeloxifene (INN for centchroman<sup>[6]</sup>) was synthesized<sup>[11]</sup> and stored as directed. All other chemicals were of analytical grade.

### Animals and treatment

Rats were randomized into 19 groups of ten each and kept in plastic cages containing dry rice husk. Animals received either ormeloxifene or raloxifene (0.25, 1.25, or 3 mg/kg) once daily for 7, 15, or 30 days by the oral route. Animals of the control group received the vehicle (gum-acacia in distilled water) alone in a similar manner. Ormeloxifene and raloxifene were individually macerated with almost equal quantity of gum-acacia and suspended in distilled water to achieve desired concentrations such that each rat received 0.2 ml of freshly prepared suspension/100 g body weight.<sup>[7]</sup> Doses used included contraceptive dose (0.25 mg/kg/day)<sup>[12]</sup> of ormeloxifene and dose of ormeloxifene (1.25 mg/kg/day)<sup>[7]</sup> and raloxifene (3 mg/kg/day)<sup>[13]</sup> required to preserves bone mass in ovariectomized rats. Twenty-four-hours after the last treatment, trachea of each rat, maintained on a heating table (37 °C; HugoSachs Electronic, Germany), was cannulated under pentobarbital anesthesia.<sup>[14]</sup> Intermittent positive pressure respiration with oxygen-atmospheric air mixture (2.5 ml, 120/min) was maintained using Animal Ventilator (Harvard Apparatus, USA).

### Hemodynamics

Common carotid arteries were dissected free and mean blood velocity was measured in right carotid artery with

20 MHz pulsed-Doppler flow probe (CBI-8000; Crystal Biotech, Hopkinton).<sup>[15]</sup> Left carotid artery was cannulated with heparinized (50 U heparin/ml physiological saline) polyethylene cannula connected to pressure transducer for measurement of systolic and diastolic pressure using data acquisition system (Biopac Systems Inc., USA) and mean arterial blood pressure was calculated.<sup>[16]</sup> Electrocardiogram was recorded using ECG-100C (NICO-100C, Biopac Systems Inc., USA). Monitoring electrodes placed subcutaneously on lower chest were connected to transducers and amplifiers. After 5 min equilibration, hemodynamic parameters were recorded for 20 min. One milliliter blood was collected from carotid artery of each rat via catheter for antioxidant activity estimation.

### Coagulation parameters

Coagulation parameters were estimated only in rats treated with 3 mg/kg/day dose of ormeloxifene or raloxifene for 30 days. Thrombin time (TT), prothrombin time (PT), and activated partial thromboplastin time (aPTT) were assessed<sup>[17]</sup> in platelet poor plasma (PPP) using semi-automated coagulometer (Start4, Young Instruments, France) and STA thrombin reagent, Neoplastin CI plus, Fibrin-Prest and CK Prest, respectively, according to the manufacturer's instructions. For PPP, blood collected by cardiac puncture using syringe containing 3.2% trisodium citrate was centrifuged at  $2500 \times g$  for 15 min at 20°C.

### Antioxidant activity

Serum total antioxidant activity (AOA) was estimated according to Koracevic *et al.*<sup>[18]</sup> Uric acid (1 mmol/l in 5 mmol/l NaOH) was used as standard.

### Statistical analysis

Comparisons between different groups were made using two-way ANOVA with Newman-Keuls multiple comparison test. Microcal software version 6.0 was used for data analysis.<sup>[19]</sup> A *P* value < 0.05 was considered significant.

## RESULTS

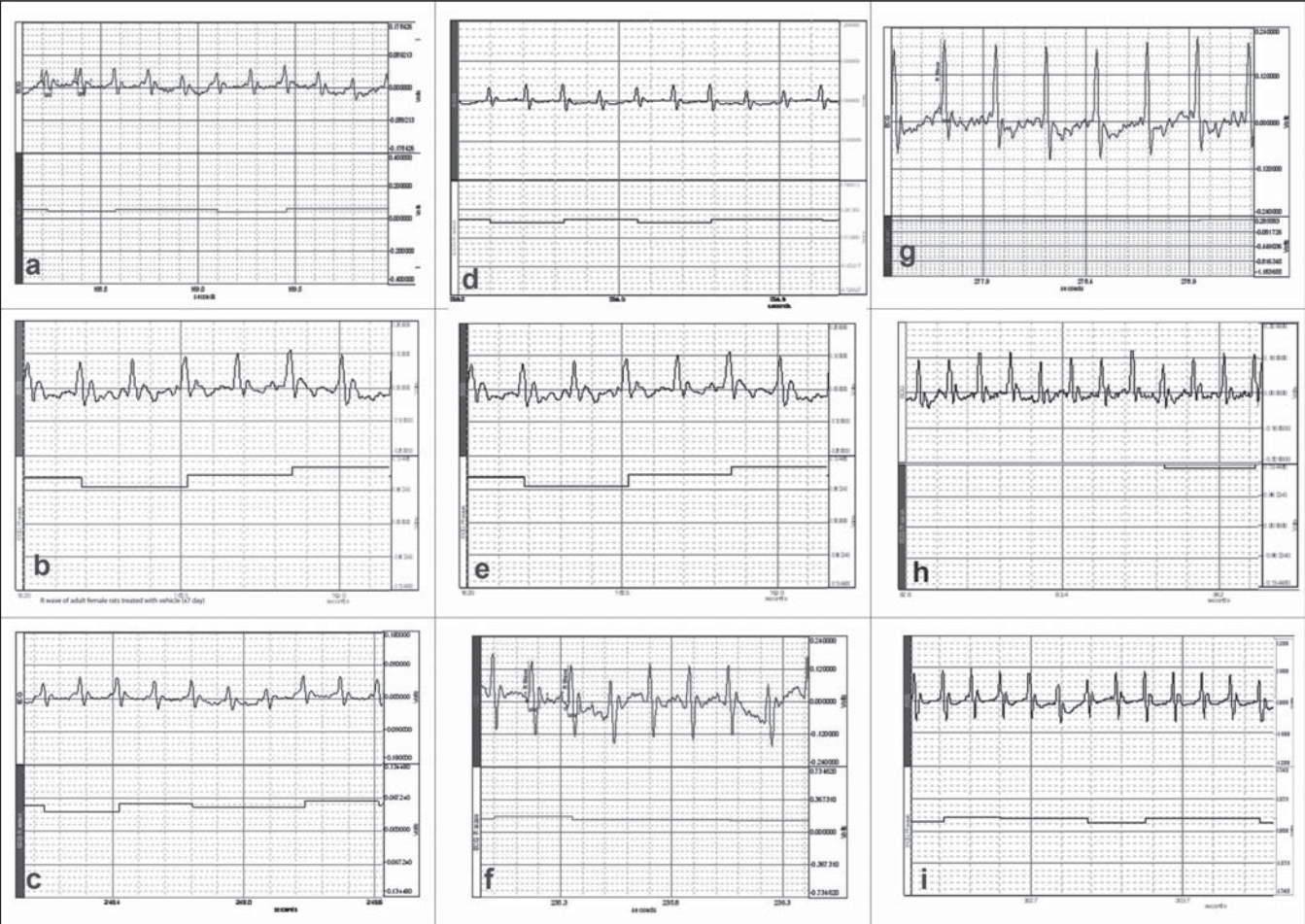
### Hemodynamic parameters

Both ormeloxifene and raloxifene administered up to 3 mg/kg daily dose for up to 30 days did not significantly alter any hemodynamic parameters, viz., heart rate (vehicle control group:  $337.0 \pm 11.4$  beats/min), mean blood velocity (vehicle control group:  $17.2 \pm 1.4$  cm/sec), systolic, diastolic, and mean blood pressure (vehicle control group:  $152.3 \pm 4.7$ ,  $112.3 \pm 3.9$ , and  $125.7 \pm 3.8$ , respectively) in adult female rats. There was also no observable effect on different parameters of heart [Table 1], except that both ormeloxifene (3 mg/kg  $\times$  30 days) and raloxifene (3 mg/kg  $\times$  7 days) significantly (*P* < 0.05) increased amplitude of R wave signal [Figure 1]. In rats treated with 0.25 and 1.25 mg/kg/day doses of ormeloxifene

**Table 1: Effect of ormeloxifene and raloxifene on electrocardiogram in adult female rats**

| Treatment    | Dose (mg/kg) | Days of treatment | R wave (V)               | R–R (s)     | QT (s)       |
|--------------|--------------|-------------------|--------------------------|-------------|--------------|
| Vehicle      | --           | 30                | 0.14 ± 0.02              | 0.42 ± 0.03 | 0.08 ± 0.003 |
| Ormeloxifene | 0.25         | 7                 | 0.16 ± 0.03              | 0.50 ± 0.05 | 0.07 ± 0.003 |
|              |              | 15                | 0.08 ± 0.02              | 0.35 ± 0.01 | 0.08 ± 0.002 |
|              |              | 30                | 0.12 ± 0.01              | 0.36 ± 0.01 | 0.08 ± 0.002 |
| Raloxifene   | 0.25         | 7                 | 0.09 ± 0.01              | 0.36 ± 0.01 | 0.06 ± 0.001 |
|              |              | 15                | 0.10 ± 0.01              | 0.40 ± 0.01 | 0.07 ± 0.001 |
|              |              | 30                | 0.14 ± 0.02              | 0.40 ± 0.01 | 0.08 ± 0.007 |
| Ormeloxifene | 1.25         | 7                 | 0.19 ± 0.03              | 0.40 ± 0.01 | 0.08 ± 0.002 |
|              |              | 15                | 0.13 ± 0.04              | 0.40 ± 0.02 | 0.08 ± 0.001 |
|              |              | 30                | 0.12 ± 0.02              | 0.34 ± 0.02 | 0.09 ± 0.001 |
| Raloxifene   | 1.25         | 7                 | 0.09 ± 0.02              | 0.36 ± 0.01 | 0.08 ± 0.001 |
|              |              | 15                | 0.20 ± 0.08              | 0.37 ± 0.08 | 0.07 ± 0.001 |
|              |              | 30                | 0.18 ± 0.01              | 0.37 ± 0.02 | 0.08 ± 0.002 |
| Ormeloxifene | 3            | 7                 | 0.16 ± 0.02              | 0.40 ± 0.01 | 0.09 ± 0.001 |
|              |              | 15                | 0.10 ± 0.02              | 0.39 ± 0.01 | 0.08 ± 0.002 |
|              |              | 30                | 0.24 ± 0.02 <sup>a</sup> | 0.40 ± 0.01 | 0.07 ± 0.001 |
| Raloxifene   | 3            | 7                 | 0.26 ± 0.01 <sup>a</sup> | 0.40 ± 0.01 | 0.08 ± 0.002 |
|              |              | 15                | 0.14 ± 0.01              | 0.37 ± 0.01 | 0.07 ± 0.002 |
|              |              | 30                | 0.18 ± 0.02              | 0.37 ± 0.01 | 0.07 ± 0.002 |

R wave: Amplitude of R wave, R–R: R–R interval or interval between two R peaks, QT: QT interval or distance between beginning of Q wave and end of T wave, Values are mean ± SEM, <sup>a</sup>*P* < 0.05, *versus* corresponding vehicle control group, All other relevant comparisons were statistically nonsignificant.



**Figure 1:** R wave of adult female rats treated with vehicle (a, b, c), ormeloxifene (3 mg/kg/day; d, e, f), or raloxifene (3 mg/kg/day; g, h, i) for 7, 15, or 30 days, respectively. Significant (*P* < 0.05) increase in amplitude of R wave was observed in rats treated with 3 mg/kg/day dose of ormeloxifene for 30 days, which in raloxifene treated rats was evident only 7 days after treatment at this dose



for 30 days, the amplitude of R wave was  $0.12 \pm 0.01$  and  $0.12 \pm 0.02$  V, respectively, which was not significantly different from controls [ $0.14 \pm 0.02$  V, Table 1]. In rats treated with 3 mg/kg daily dose of ormeloxifene for 30 days, the amplitude of R wave ( $0.24 \pm 0.02$  V) increased by 71.4% ( $P < 0.05$ ) as compared to vehicle control level. In rats treated with 0.25 and 1.25 mg/kg daily doses of raloxifene for 7 days, the amplitude of R wave was  $0.09 \pm 0.01$  and  $0.09 \pm 0.02$  V, respectively. In comparison, in rats treated with 3 mg/kg daily dose of raloxifene for 7 days, the amplitude of R wave ( $0.26 \pm 0.01$  V) was increased by 85.7% ( $P < 0.05$ ). This effect of raloxifene was not manifested when duration of treatment was increased to 15 or 30 days.

### Coagulation parameters

No significant change was observed in coagulation profile (thrombin time, prothrombin time, or activated partial thromboplastin time; mean  $\pm$  SEM in seconds) of animals after administration of either drug (vehicle versus ormeloxifene - TT:  $48.5 \pm 7.1$  versus  $49.5 \pm 6.5$ ; PT:  $18.8 \pm 3.2$  versus  $17.6 \pm 0.9$ ; aPTT:  $16.7 \pm 0.3$  versus  $16.2 \pm 0.9$ ; vehicle versus raloxifene - TT:  $32.6 \pm 3.3$  versus  $34.3 \pm 5.1$ ; PT:  $14.4 \pm 0.4$  versus  $15.1 \pm 0.3$ ; aPTT:  $17.0 \pm 0.2$  versus  $16.8 \pm 1.2$ ) at the highest (3 mg/kg/day) dose administered for the longest duration of observation (i.e., 30 days).

### Antioxidant activity

There was no significant difference in serum total AOA (mmol/l; mean  $\pm$  SEM) in rats receiving ormeloxifene or raloxifene at these doses and schedules. Apparent increase in AOA in rats receiving ormeloxifene [0.25 ( $1.89 \pm 0.07$ ), 1.25 ( $1.87 \pm 0.10$ ), or 3 ( $1.70 \pm 0.07$ ) mg/kg/day for 15 days) or raloxifene (1.25 mg/kg/day for 7 days;  $1.69 \pm 0.06$ ) was statistically nonsignificant when compared to that in rats receiving vehicle alone for 30 days ( $1.39 \pm 0.09$ ).

## DISCUSSION

Results of this study demonstrate lack of effect of ormeloxifene administered at its contraceptive, antiresorptive and higher doses in different schedules on various hemodynamic parameters in adult female rats. There was also no observable effect on different heart parameters, except increase in amplitude of R wave in rats treated with the highest (3 mg/kg/day) dose of ormeloxifene for 30 days. This effect in case of raloxifene was evident only 7 days after treatment at this dose. While this might be related to stronger ventricular contractions after administration of these drugs, we did not observe any change in blood pressure or other hemodynamic parameters as a consequence of observed change in R wave in treated rats. Based on the available information, it is not possible to assign any physiological significance to increase in amplitude of R wave. Overall response was, however,

almost similar in case of both the agents. According to a recent study,<sup>[20]</sup> raloxifene (1  $\mu$ M) does not increase QRS and QTc interval in isolated guinea pig heart suggesting it to be a safe SERM. Previous studies have also demonstrated lipid lowering effect of ormeloxifene in animal and clinical settings.<sup>[5]</sup> In adult female rabbits, ormeloxifene (0.1, 1, 10 mg/kg) administered for 4 days caused 40%-70% inhibition in ovariectomy-induced increase in serum total cholesterol, without adverse effects of estrogen therapy. No increase in serum total or HDL cholesterol or triglycerides was observed in women in 30 mg/week dose phase-III multicentric trial of ormeloxifene with use duration of 1-2 years, or in lactating mothers ( $n = 4$ ).<sup>[5]</sup>

It is now well established that the relative risk of symptomatic venous thromboembolism (VTE) in healthy women associated with raloxifene use increases by 2-3-fold. Such risk translates into estimated absolute risk of 2-3 cases/1000/year,<sup>[21]</sup> which is similar to the absolute risk of VTE in women using HRT.<sup>[22]</sup> Slight reduction in antithrombin-III is reported after 3-6 months of raloxifene use.<sup>[23]</sup> In follow-up study for 8 years involving 4011 females who were treated with raloxifene for postmenopausal osteoporosis, 1.7-fold increase in risk of VTE was observed.<sup>[24]</sup> Extrapolation of available literature might suggest caution against indiscriminate use of raloxifene in women with past history of idiopathic VTE and in asymptomatic carriers of inherited hypercoagulable states.<sup>[25]</sup> We did not find any significant difference in coagulation profile in rats treated with ormeloxifene or raloxifene for 30 days. This finding, however, by no means precludes that these drugs are free from coagulation side effects.

In conclusion, the findings demonstrate comparable pharmacological profile of ormeloxifene and raloxifene in a short-term administration to rats. Based on changes observed in the ECG (R wave), long-term studies may lead to justifiable comparison of beneficial and harmful effects of ormeloxifene and raloxifene in relation to cardiovascular effects.

## ACKNOWLEDGMENTS

This work was carried out at Central Drug Research Institute, Lucknow, India. Dr. Man Mohan Singh thanks the Indian Council of Medical Research, New Delhi for award of Emeritus Scientist scheme.

## REFERENCES

1. Manson JE. Postmenopausal hormone therapy and atherosclerotic disease. *Am Heart J* 1994;128:1337-43.
2. Beresford SA, Weiss NS, Voigt LF, McKnight B. Risk of endometrial cancer in relation use of oestrogen combined with cyclic progestagen therapy in postmenopausal women. *Lancet* 1997;349:458-61.
3. Riggs L, Hartmann LC. Selective estrogen receptor modulators-mechanisms of action and application to clinical practice. *N Engl J Med* 2003;348:618-29.

# Bhalla, *et al.*: Effect of ormeloxifene on systemic hemodynamics

4. Delmas PD. Treatment of postmenopausal osteoporosis. *Lancet* 2002;359:2018-26.
5. McDonnell DP, Connor CE, Wijayarathne A, Chang C, Norris JD. Definition of the molecular and cellular mechanisms underlying tissue-selective agonist/antagonist activities of selective estrogen receptor modulators. *Recent Progress Horm Res* 2002;57:295-316.
6. Singh MM. Ormeloxifene, a selective estrogen receptor modulator, as contraceptive and in the management of hormone related clinical disorders. *Med Res Rev* 2001;21:302-47.
7. Narayana Murthy PS, Sengupta S, Sharma S, Singh MM. Ormeloxifene prevents ovariectomy-induced bone loss by inhibition of osteoclastogenesis, apoptosis of mature osteoclasts and up-regulation of TGF $\beta$ -3 expression in rat. *J Steroid Biochem Mol Biol* 2006;100:117-28.
8. Hall TJ, Nyugen H, Schaublin M, Fournier B. Bone specific estrogen centchroman inhibits osteoclastic bone resorption *in vitro*. *Biochem Biophys Res Commun* 1995;216:662-8.
9. Srivastava R, Puri V, Srimal RC, Dhawan BN. Prostanoid mediated effects of centchroman, a nonsteroidal oral contraceptive. *Agents Actions* 1986;18:596-9.
10. Black LJ, Sato M, Rowley ER, Magee DE, Bekele A, Williams DC, *et al.* Raloxifene prevents bone loss and reduces serum cholesterol without causing uterine hypertrophy in ovariectomized rats. *J Clin Invest* 1994;93:63-9.
11. Saeed A, Sharma AP, Durani N, Jain R, Durani S, Kapil RS. Structure-activity relationship of antiestrogens: Studies on 2,3-diaryl-1-benzopyrans. *J Med Chem* 1990;33:3210-6.
12. Singh MM, Kamboj VP. Fetal resorption in rats treated with an antiestrogen in relation to luteal phase nidatory estrogen secretion. *Acta Endocrinol* 1992;26:444-50.
13. Turner CH, Sato M, Bryant HU. Raloxifene preserves bone strength and bone mass in ovariectomized rats. *Endocrinology* 1994;135:2001-5.
14. Li Y, Wang L, Schuschke DA, Zhou Z, Saari JT, Kang YJ. Marginal dietary copper restriction induces cardiomyopathy in rats. *J Nutrition* 2005;135:2130-6.
15. Gardiner SM, Compton AM, Bennett T, Hartley CJ. Can pulsed Doppler technique measure changes in aortic blood flow in conscious rats? *Am J Physiol* 1990;259:H448-56.
16. Gevers M, Hack MW, vanGenderingen HR, Lafeber HN, Westerhof N. Calculated mean arterial pressure in posterior tibial and radial artery pressure wave in newborn infants. *Basic Res Cardiol* 1995;90:247-51.
17. Batra S, Roy AK, Patra A, Bhaduri AP, Surin WR, Raghavan SA, *et al.* Baylis-Hillman reaction assisted parallel synthesis of 3,5-disubstituted isoxazoles and their *in vivo* bioevaluation as antithrombotic agents. *Bioorg Med Chem* 2004;12:2059-77.
18. Koracevic D, Koracevic G, Djordjevic V, Andrejevic S, Cosic V. Method for measurement of antioxidant activity in human fluids. *J Clin Pathol* 2001;54:356-61.
19. Decher N, Uyguner O, Scherer CR, Karaman B, Yüksel-Apak M, Busch AE, *et al.* hKChIP2 is a functional modifier of hKv4.3 potassium channels: Cloning and expression of a short hKChIP2 splice variant. *Cardiovas Res* 2001;52:255-64.
20. Liu H, Yang L, Jin M-W, Sun H-Y, Huang Y, Li G-R. Selective estrogen receptor modulator raloxifene inhibits cardiac delayed rectifier potassium currents and voltage-gated sodium current without QTc interval prolongation. *Pharmacol Res* 2010;62:384-90.
21. Cauley JA, Norton L, Lippman ME, Eckert S, Krueger KA, Purdie DW, *et al.* Continued breast cancer risk reduction in postmenopausal women treated with raloxifene: 4-Year results from MORE trial. *Breast Cancer Res Treatment* 2001;65:125-34.
22. Grady D, Wenger NK, Herrington D, Khan S, Furberg C, Hunninghake D, *et al.* Postmenopausal hormone therapy increases risk for venous thromboembolic disease: Heart and estrogen/progestin replacement study. *Ann Intern Med* 2000;132:689-96.
23. Azevedo GD, Franco RF, Baggio MS, Maranhão TM, Ferriani RA, Silva de Sá ME. Effects of raloxifene therapy on anticoagulant system in postmenopausal women. *Climacteric* 2003;6:140-5.
24. Martino S, Disch D, Dowsett SA, Keech CA, Mershon JL. Safety assessment of raloxifene over eight years in a clinical trial setting. *Curr Med Res Opin* 2005;21:1441-52.
25. Hoibraaten E, Qvigstad E, Arnesen H, Larsen S, Wickstrom E, Sandset PM. Increased risk of recurrent venous thromboembolism during hormone replacement therapy - Results of the randomized, double-blind, placebo-controlled Estrogen in Venous Thromboembolism Trial. *Thrombosis Haemostasis* 2000;84:961-7.

**How to cite this article:** Bhalla H, Pant KK, Dikshit M, Surin WR, Singh MM. Effect of ormeloxifene, a nonsteroidal once-a-week oral contraceptive, on systemic hemodynamics in adult female rats. *J Pharmacol Pharmacother* 2011;2:90-4

**Source of Support:** Nil, **Conflict of Interest:** None declared.

## Author Help: Reference checking facility

The manuscript system ([www.journalonweb.com](http://www.journalonweb.com)) allows the authors to check and verify the accuracy and style of references. The tool checks the references with PubMed as per a predefined style. Authors are encouraged to use this facility, before submitting articles to the journal.

- The style as well as bibliographic elements should be 100% accurate, to help get the references verified from the system. Even a single spelling error or addition of issue number/month of publication will lead to an error when verifying the reference.
- Example of a correct style  
Sheahan P, O'leary G, Lee G, Fitzgibbon J. Cystic cervical metastases: Incidence and diagnosis using fine needle aspiration biopsy. *Otolaryngol Head Neck Surg* 2002;127:294-8.
- Only the references from journals indexed in PubMed will be checked.
- Enter each reference in new line, without a serial number.
- Add up to a maximum of 15 references at a time.
- If the reference is correct for its bibliographic elements and punctuations, it will be shown as CORRECT and a link to the correct article in PubMed will be given.
- If any of the bibliographic elements are missing, incorrect or extra (such as issue number), it will be shown as INCORRECT and link to possible articles in PubMed will be given.